THE DELAYED OCCURRENCE AND TOTAL OMISSION OF ENDOMIXIS IN SELECTED LINES OF PARAMECIUM AURELIA

T. M. SONNEBORN

(*From the Department of Zoölogy, Johns Hopkins University*)

Experimental control of the process of endomixis in *Paramecium aurelia* has been only partly achieved. Jollos (1916), Sonneborn (1937*b*) and others have developed methods of inducing endomixis, but no method of avoiding it completely is known. Temporary suppression of endomixis was probably attained by Jollos (1916); but the method employed volumes of culture medium so great as to make it impossible to ascertain with reliability whether endomixis was occurring or not. The present paper sets forth a method that avoids this difficulty and provides not only some lines of descent with greatly extended interendomictic intervals, but also others that never go into endomixis.

The method is based on the observation (Sonneborn, 1937*a*) that the interendomictic interval varies greatly in sister lines cultivated under the same conditions. An attempt was therefore made to select the lines with the longer intervals. This was done by discarding lines as they went into endomixis and replacing them by new lines begun with surplus individuals from sister lines that had not yet gone into endomixis. When this is done with a group of 12 to 24 daily isolation lines cultivated under the conditions employed by Sonneborn (1936), the following phenomena occur. At first, none of the lines go into endomixis; then, as the "normal" time for endomixis approaches, more and more of the lines go into endomixis and have to be replaced by their sister lines that have not yet gone into endomixis. During a period of several weeks, this high frequency of endomixis continues and necessitates equally frequent eliminations and replacements; but during the following few weeks the frequency of endomixis greatly decreases and thereafter occurs but rarely. Selection has thus been effective in seeking out lines in which endomixis completely fails to occur. However, such lines cannot be continued indefinitely; after four or five months they die.

This method has been successfully applied in this laboratory many times and is now a routine technique. As illustrations of the results obtainable with it, two typical histories will be given in the following

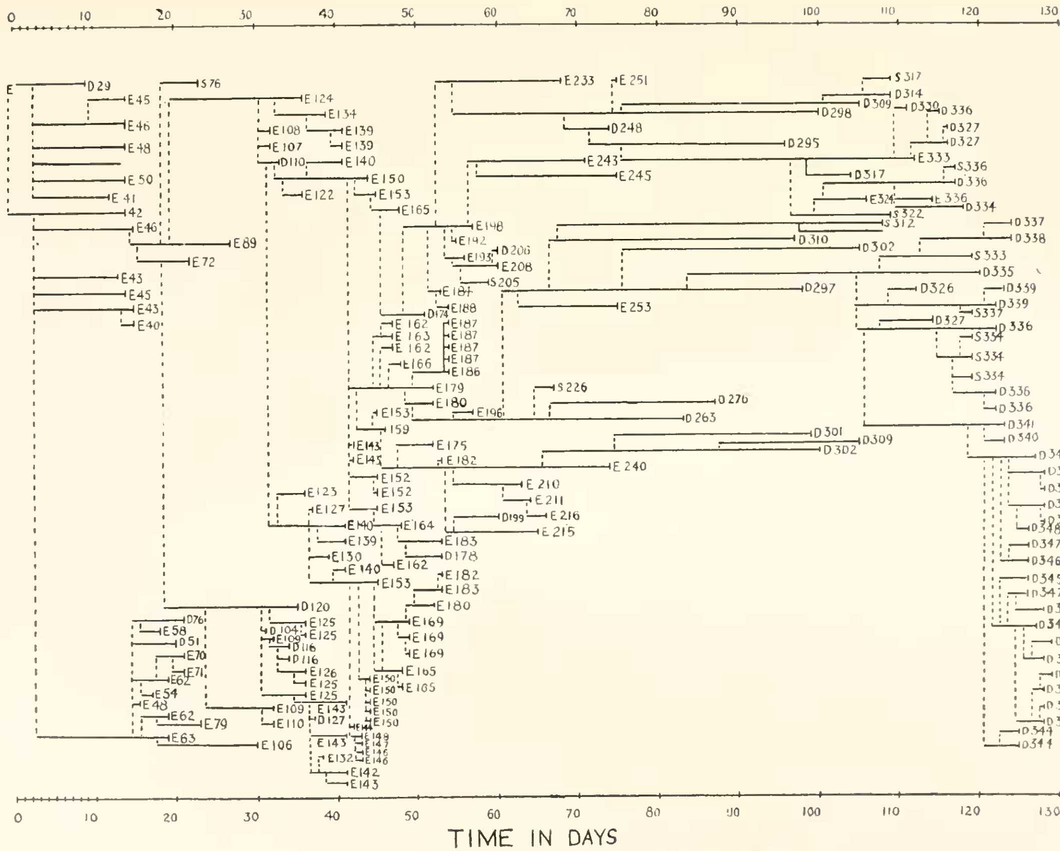

FIG. 1. Selection of non-endomictic lines in race *R*, *Paramecium aurelia*. The solid horizontal lines represent daily isolation lines of descent. The broken vertical lines connect sub-cultures with their source cultures. *E* symbolizes endomixis; *D*, died; and *S*, killed and stained after two days without fission. The numbers following *E*, *D*, and *S* give the number of fissions without endomixis in the direct line of descent from the original progenitor in endomixis on the first day to the final individual which terminated each sub-culture.

two sections: one of these involves animals of the Johns Hopkins race *R*, reported upon in recent papers by the author and associates; the other involves animals of Woodruff's long-lived race, here designated *W*. In the third section, the relation of these results to the variability of the interendomictic interval is set forth.

Race R

Figure 1 gives the results of applying the method of selection to a group of typical lines of race *R*. The entire group was derived from one individual in endomixis April 14, 1935. The original 12 lines descended from this individual went into endomixis between the 14th and 20th days (after 41 to 63 fissions) following the initial endomixis.

Table I

The frequency of endomixis and of death in relation to the time since the last preceding endomixis in races R and W of *Paramecium aurelia*

Race	Days since preceding endomixis	Number of lines in endomixis per 100 line-days	Number of deaths per 100 line-days
R	1–13	0	0.8
	14–55	23.3	2.6
	56–76	6.4	2.4
	77–122	0.6	5.9
	123–130	0	35.8
W	1–30	0	2.0
	31–76	3.8	0.8
	77–130	0.5	4.8
	131–163	0	13.4

Each of these lines was replaced at the time of endomixis by a new line started with a surplus individual from one of the sister lines that had not yet gone into endomixis. By repeating this process of replacement in all the descendant lines whenever endomixis occurred or might have occurred, some of the lines lived for as much as 130 days (350 fissions) without endomixis.

As shown in Table I, the life of this group of lines fell into five periods differing markedly in the frequency of endomixis and death. The first period, extending to the 13th day, was characterized by the complete absence of endomixis. In the second period, from the 14th to 55th days, many lines went into endomixis and had to be replaced. The third period, from the 56th to 76th day, was marked by a great reduction of the number of lines going into endomixis. In the fourth period, from the 77th to 122nd day, only three lines went into endomixis, but the number that died or stopped multiplying increased. Finally, in the fifth period, from the 123rd to 130th day, no line went into endomixis, but the death rate was so high that the group completely died out. In Table I, lines which stopped multiplying and were found, on staining, not to be in endomixis, are included among those which died. Experience showed that this was their usual fate.

The main feature of the preceding account is that endomixis did not occur in certain lines of descent carried through 350 fissions during 130 days. This was demonstrated by cytological studies. Each line of descent was stained on every day that fission occurred; and if fission failed to occur in any line for two successive days the line was stained and so brought to an end. Since every line that showed any nuclear condition even remotely suggesting endomixis was discarded, it is certain that no endomixis was overlooked and that the lines living through till the end of the experiment had not experienced endomixis during the entire time.

Race *W*

The method of obtaining non-endomictic lines of descent was also applied successfully to race *W*, though here the detailed results differ from those obtained with race *R*. A typical set of results on race *W* is illustrated by Fig. 2. The group there represented began with four individuals in endomixis on February 10, 1935. As shown in Table I, the life of this group is divisible into four distinct periods. In the first period, extending to the 30th day, there was no endomixis and few deaths occurred; in the second period, from the 31st to the 76th day, the frequency of endomixis was at its highest and the death rate was still low; in the third period, from the 77th to 130th day, there were few endomixes (two certainly, and possibly four), but the death rate showed a marked increase; in the fourth period, from the 131st to 163rd day, there were no further endomixes and the death rate reached its peak, resulting in the extermination of the group.

As in race *R*, the lines of race *W* which were carried to the end of the experiment showed no endomixis during the entire period of observation. Their life without endomixis extended for 163 days and included 302 successive fissions. This long period is $5\frac{1}{2}$ times as long as those reported to be typical for this race by Woodruff and Erdmann (1914) and it includes about seven times as many fissions.

Comparison of the data for the two races given in Table I shows that the group of race *W* lines had a longer initial period without endomixis (30 days as compared with 13), and that it at no time attained so high a rate of endomixis or death as did the group of race *R* lines. Further, the maximum period without endomixis was longer in race *W* than in race *R*, but included fewer fissions.

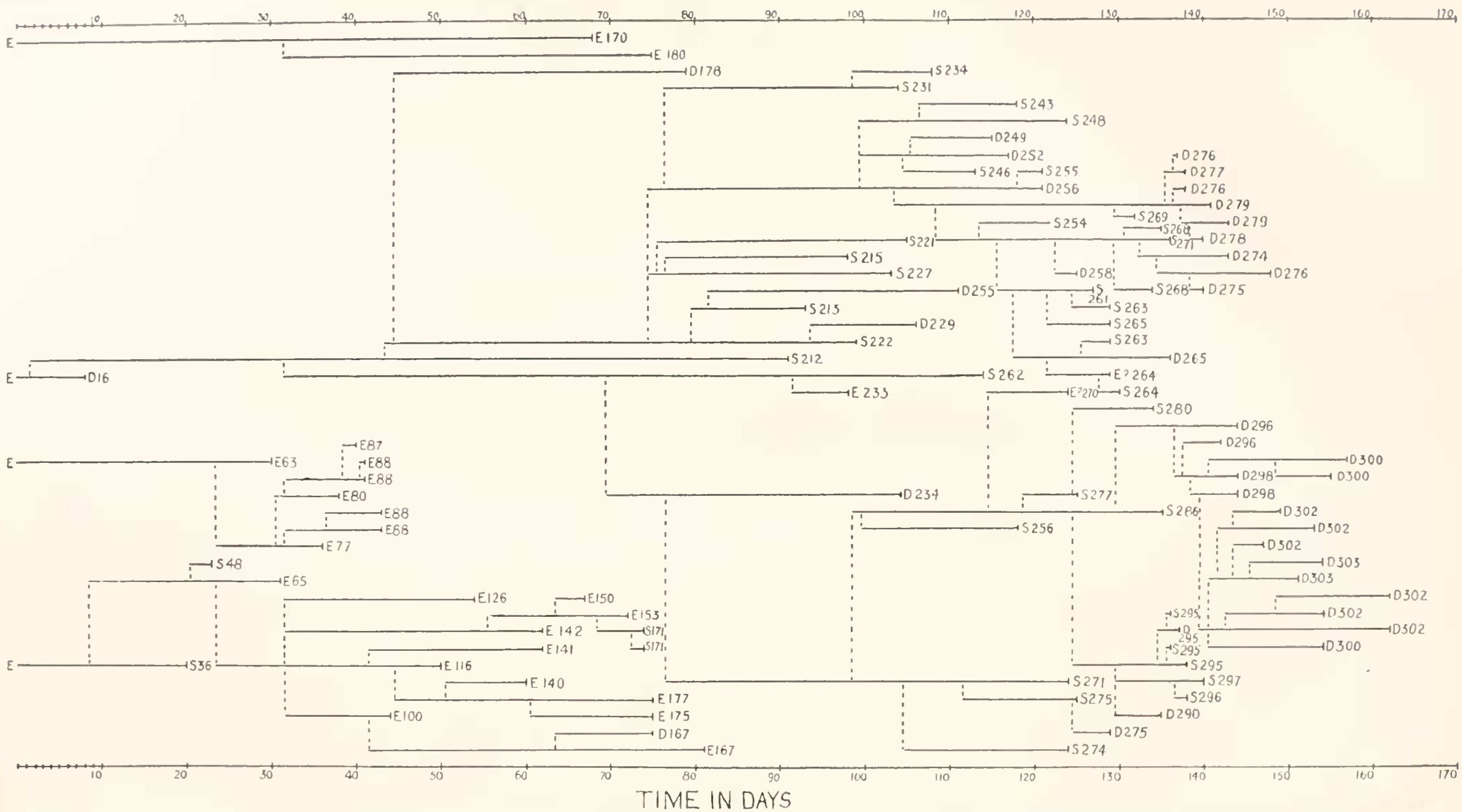

FIG. 2. Selection of non-endomictic lines in race *W*, *Paramecium aurelia*. The solid horizontal lines represent daily isolation lines of descent. The broken vertical lines connect sub-cultures with their source cultures. *E* symbolizes endomixis; *D*, died; and *S*, killed and stained after two days without fission. The numbers following *E*, *D*, and *S* give the number of fissions without endomixis in the direct line of descent from the original progenitors in endomixis on the first day to the final individual which terminated each sub-culture.

Three other races (*H*, *E*, and *S*) have been subjected to the same method of selection with similar results. It seems reasonable to suppose that what has been accomplished in these five races could probably be done with most or all races of *P. aurelia* under similar cultural conditions: by selection, lines can be isolated that do without endomixis throughout their lives—a period 5 to 7 times as long as the ordinary interval between successive endomixes.

Variability of the Interendomictic Interval

In the absence of selection, the extent of the period during which no nuclear reorganization occurs was shown by Sonneborn (1937*a*) to vary greatly. The preceding sections show that still greater variability appears when the selection technique is applied. This greater variability must be due to the existence of relatively rare lines which, without any special experimental treatment, have extremely long interendomictic intervals or even fail completely to undergo endomixis. Since such lines are very rare, they would ordinarily not be found in work where the investigator is able to observe only a small sample of a race. The method of selection serves simply to seek out these lines and multiply them. Without resorting to any change of cultural conditions, it provides a means of obtaining both lines that long omit endomixis and lines that omit it entirely.

Summary

If daily isolation lines of *P. aurelia* are regularly discarded as soon as they go into endomixis, and if these are then replaced by sister lines that have not yet gone into endomixis, it is possible to maintain for long periods lines which have not been in endomixis since the start of this procedure. With this method there were obtained lines of race *R* which omitted endomixis for as long as 130 days and 350 fissions, and lines of race *W* which omitted endomixis for 163 days and 303 fissions. At the end of these long periods, all lines died.

In the culture of groups of lines selected in this way, there is an initial period during which endomixis does not occur; this is followed by a period in which endomixis occurs in many of the lines (which have to be discarded); during the remaining history, endomixis occurs rarely and eventually not at all, but the death rate rises and results in the final extinction of the group. The details as to the duration of these periods and their characteristic death and endomixis rates differ in different races. The method of selection emphasizes the enormous variability of the interendomictic interval. The extremely long intervals are very rare, and the selection method is simply a device for finding these rare lines and multiplying them.

LITERATURE CITED

JOLLOS, V., 1916. Die Fortpflanzung der Infusorien und die potentielle Unsterblichkeit der Einzelligen. *Biol. Centralbl.*, **36**: 497.

SONNEBORN, T. M., 1936. Factors determining conjugation in Paramecium aurelia. I. The cyclical factor: The recency of nuclear reorganization. *Genetics*, **21**: 503.

SONNEBORN, T. M., 1937*a*. The extent of the interendomictic interval in Paramecium aurelia and some factors determining its variability. *Jour. Exper. Zool.*, **75**: 471.

SONNEBORN, T. M., 1937*b*. Induction of endomixis in Paramecium aurelia. *Biol. Bull.*, **72**: 196.

WOODRUFF, L. L. AND R. ERDMANN, 1914. A normal periodic reorganization process without cell fusion in Paramecium. *Jour. Exper. Zoöl.*, **17**: 425.